

What is Claimed is:

1. A method for determining the presence of a specific nucleotide sequence in an RNA reagent of a target sample, said method comprising the steps of:

5        a) incubating a mixture comprising:

(i) a first component including an RNA reagent extracted directly from a target sample, said RNA reagent having a target nucleotide sequence and a capture sequence, and

10        (ii) a second component comprising a capture reagent having at least one first arm containing a label capable of emitting a detectable signal and at least one second arm having a nucleotide sequence complementary to the capture sequence of the RNA reagent of the first component,

15        at a first temperature and for a time sufficient to induce the capture sequence of the RNA reagent of the first component to bind to the complementary nucleotide sequence of the capture reagent of the second component, and thereby form a pre-hybridized RNA-capture reagent complex comprising the target nucleotide sequence;

20        b) contacting the pre-hybridized RNA-capture reagent complex with a microarray having thereon a plurality of features each containing a particular probe nucleotide sequence; and

25        c) incubating the pre-hybridized RNA-capture reagent complex on the microarray at a second temperature and for a time sufficient to hybridize the target nucleotide sequence of the pre-hybridized RNA-capture reagent complex to the complementary probe nucleotide sequence contained within the feature, wherein the

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T  
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*SUB  
A'  
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presence of such hybridization results in the emission of the detectable signal from the corresponding feature, and the absence thereof results in no emission of the detectable signal from the corresponding feature, thus generating a detectable hybridization pattern for subsequent analysis.

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2. The method of claim 1 wherein the capture reagent is selected from the group consisting of dendrimers, carbohydrates, proteins, and nucleic acids.

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3. The method of claim 2 wherein the capture reagent is a dendrimer.

4. The method of claim 1 further comprising passing a base solution over the microarray to separate and purge the hybridized RNA reagent from the probe nucleotide sequence for enabling reuse of the microarray.

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5. The method of claim 4 wherein the base solution is passed over the microarray at a temperature of from about 50° to 60° C.

6. The method of claim 4 wherein the base solution is 0.05 M sodium hydroxide.

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7. The method of claim 1 wherein the capture sequence of the RNA reagent is a single-stranded oligonucleotide consisting of at least one adenine base.

8. The method of claim 7 wherein the nucleotide sequence complementary to the capture sequence is a single-stranded oligonucleotide consisting of at least one thymine base.

5 9. The method of claim 1 wherein the RNA reagent and the capture reagent are incubated at the first temperature of from about 45° to 60 ° C.

10. The method of claim 9 wherein the RNA reagent and the capture reagent are incubated for a sufficient time ranging from about 15 minutes to 24  
10 hours.

11. The method of claim 1 wherein the pre-hybridized RNA/capture reagent complex is incubated on the microarray at the second temperature of from about 45° to 65 °C.

15 12. The method of claim 11 wherein the pre-hybridized RNA/capture reagent complex are incubated on the microarray for the sufficient time ranging from about 15 minutes to 24 hours.

20 13. The method of claim 11 wherein the probe nucleotide sequences of the microarray comprises cDNA.

14. The method of claim 11 wherein the second temperature is from about 60° to 65 °C.

15. The method of claim 11 wherein the probe nucleotide sequences of the microarray comprises oligonucleotides.

5 16. The method of claim 14 wherein the second temperature is from about 45° to 55°C.

17. The method of claim 1, after incubating the pre-hybridized RNA/recapture reagent complex on the microarray, further comprising washing any  
10 free unhybridized RNA/capture reagent complex from the microarray.

18. The method of claim 1, after incubation of the first and second components, further comprising adding blocking nucleic acids to the mixture of the first and second components.

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19. A method for determining the presence of a specific nucleotide sequence in an RNA reagent of a target sample, said method comprising the steps of:

a) contacting a first component including an RNA reagent extracted  
20 directly from a target sample, said RNA reagent having a target nucleotide sequence and a capture sequence with a microarray having thereon a plurality of features each containing a particular probe nucleotide sequence;

b) incubating the RNA reagent and the complementary probe nucleotide sequences on the microarray at a first temperature and for a time sufficient to

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hybridize the target nucleotide sequence of the RNA reagent to the complementary probe nucleotide sequence contained within the feature;

c) contacting a second component comprising a capture reagent having

at least one first arm containing a label capable of emitting a detectable signal and at

5 least one second arm having a nucleotide sequence complementary to the capture sequence of the RNA reagent of the first component; and

d) incubating the capture reagent and the capture sequence of the RNA

reagent at a second temperature and for a time sufficient to induce the capture

sequence of the RNA reagent of the first component to hybridize to the

10 complementary nucleotide sequence of the capture reagent of the second

component, wherein the presence of the hybridization results in the emission of the

detectable signal from the corresponding feature, and the absence thereof results in

no emission of the detectable signal from the corresponding feature, thus generating

a detectable hybridization pattern for subsequent analysis.

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20. The method of claim 19 wherein the capture reagent is selected from the group consisting of dendrimers, carbohydrates, proteins, and nucleic acids.

21. The method of claim 20 wherein the capture reagent is a dendrimer.

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22. The method of claim 19 further comprising passing a base solution over the microarray to separate and purge the hybridized RNA reagent from the probe nucleotide sequence for enabling reuse of the microarray.

23. The method of claim 22 wherein the base solution is passed over the microarray at a temperature of from about 50° to 60° C.

24. The method of claim 22 wherein the base solution is 0.05 M sodium hydroxide.

25. The method of claim 19 wherein the capture sequence of the RNA reagent is a single-stranded oligonucleotide consisting of at least one adenine base.

10 26. The method of claim 25 wherein the nucleotide sequence complementary to the capture sequence is a single-stranded oligonucleotide consisting of at least one thymine base.

15 27. The method of claim 19 wherein the RNA reagent and the complementary probe nucleotide sequences on the microarray are incubated at the first temperature of from about 45° to 65° C.

20 28. The method of claim 27 wherein the RNA reagent and the complementary probe nucleotide sequences on the microarray are incubated for a sufficient time ranging from about 15 minutes to 24 hours.

29. The method of claim 27 wherein the probe nucleotide sequences of the microarray comprises cDNA.

30. The method of claim 29 wherein the first temperature is from about 60° to 65 °C.

31. The method of claim 27 wherein the probe nucleotide sequences of the  
5 microarray comprises oligonucleotides.

32. The method of claim 31 wherein the second temperature is from about 45° to 55°C.

10 33. The method of claim 19 wherein the capture reagent and the capture sequence of the RNA reagent are incubated at a second temperature ranging from about 45° to 60°C.

15 34. The method of claim 33 wherein the capture reagent and the capture sequence of the RNA reagent are incubated for the sufficient time ranging from about 15 minutes to 24 hours.

20 35. The method of claim 19, after incubating the capture sequence of the RNA reagent and the capture reagent on the microarray, further comprising adding blocking nucleic acids to the microarray.

36. The method of claim 19, after the incubating the RNA reagent and the complementary nucleotide probes on the microarray step, further comprising

washing the microarray with a buffer solution to remove excess unhybridized RNA reagent.

37. The method of claim 1 wherein the capture sequence of the RNA  
5 reagent comprises at least one locked nucleic acid nucleotide.

38. The method of claim 19 wherein the capture sequence of the RNA  
reagent comprises at least one locked nucleic acid nucleotide.

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